SYNTHESIS AND TRANSFER OF DNA, RNA, AND PROTEIN DURING VITELLOGENESIS IN RHODNIUS PROLIXUS (HEMIPTERA)[1]

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Two tissues, the apical trophocytes and the follicular epithelium, are active in transferring yolk to the growing oocytes in the ovarioles of most insects. The telotrophic ovary of Hemiptera is particularly useful in the study of vitellogenesis because the two nutritive tissues are sufficiently set apart so that the special activity of each can be separately investigated. Of especial interest is the study of the nucleic acid and protein components of the oocyte yolk. Investigations on the origin, distribution and nature of these materials are of fundamental importance in the search toward an understanding of the biochemistry of insect embryology.

Important cytochemical studies on vitellogenesis in Hemiptera have been performed by Schrader and Leuchtenberger (1952), who showed the apparent transfer of DNA in a depolymerized form from the apical trophocytes to the oocytes in the coreid bug, *Acanthocephala;* and by Bonhag (1955), who showed the passage of nucleic acids and protein from the trophocytes to the oocytes in the ovary of the milkweed bug, *Oncopeltus*.

The autoradiographic technique has distinct advantages over the cytochemical approach in the study of a dynamic process such as the transfer of materials between tissues. Instead of being restricted to merely localizing a chemical substance in a tissue section, it becomes possible to determine its site of synthesis, and to trace its movement from one tissue to another.

The present paper attempts to define more precisely the role of the trophic tissues in the genesis of the nucleic acid and protein components of the yolk in the growing insect oocyte, by utilizing the autoradiographic technique in conjunction with cytochemistry.

Materials and Methods

Mature *Rhodnius prolixus* adult females were used exclusively in the experiments. The bugs were maintained at 28° C. in a high humidity cabinet. They were fed weekly on the shaven belly of an immobilized rabbit.

A. Histological and cytological methods

The bugs being studied were vivisected under physiological saline solution. The ovaries and portions of the midgut and ventral fat body were dissected out and

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fixed in Carnoy's (6:3:1) fixative for 45 minutes, or in alcoholic Bouin's solution for 24 hours. Mature oocytes with a well developed chorion were punctured with a fine needle in order to facilitate penetration of histological reagents.

After fixation, Carnoy-fixed tissues were washed for one hour in absolute ethanol, while Bouin-fixed tissues were washed in several changes of 80% ethanol over a three-day period. The tissues were then hydrated to distilled water via a graded series of alcohol concentrations. Wigglesworth's (1959) agar-tested

PLATE I

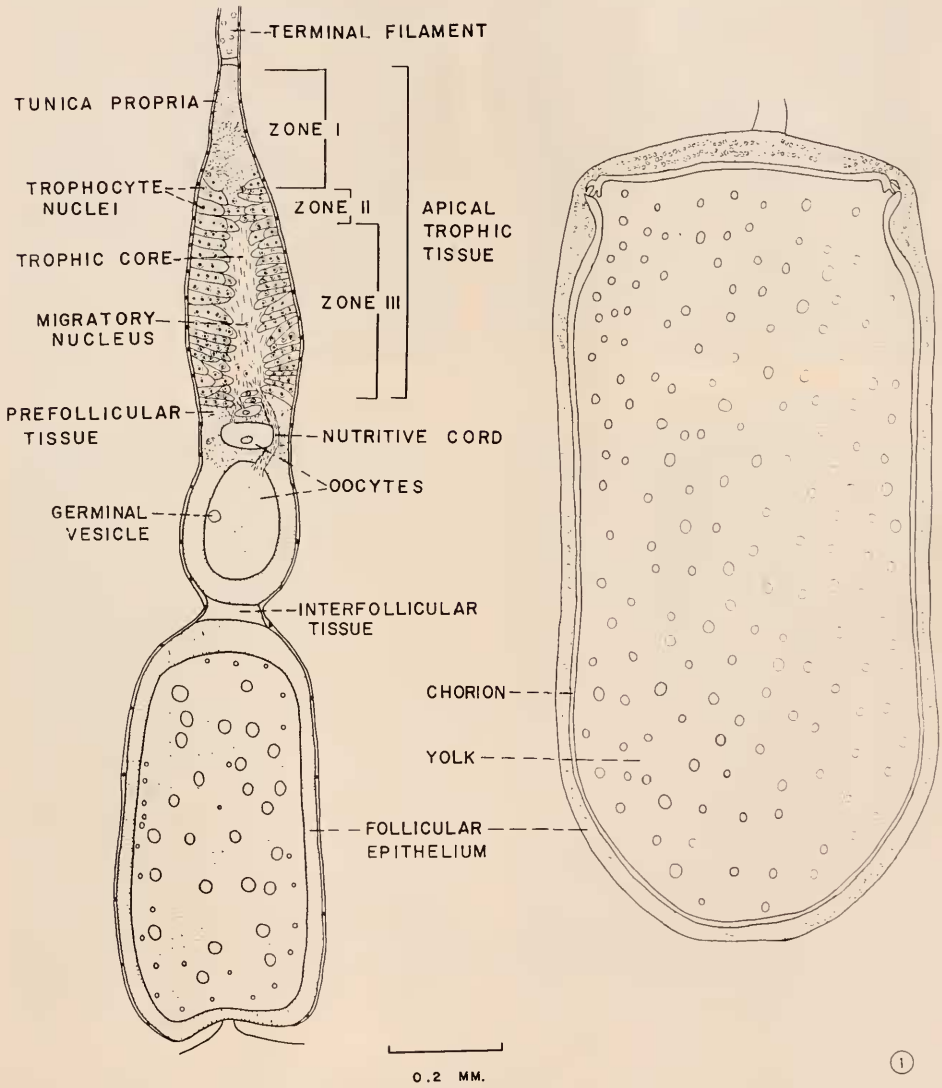
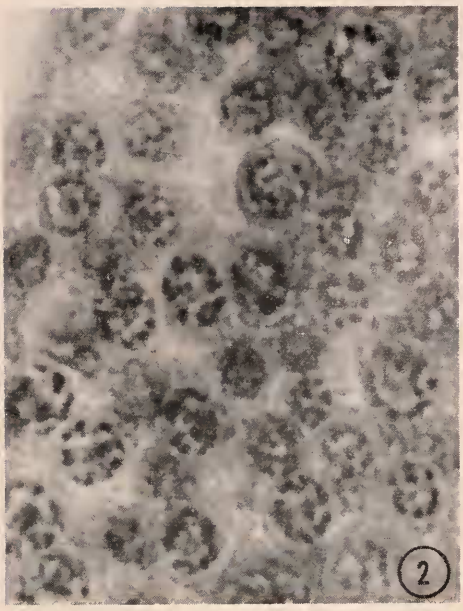
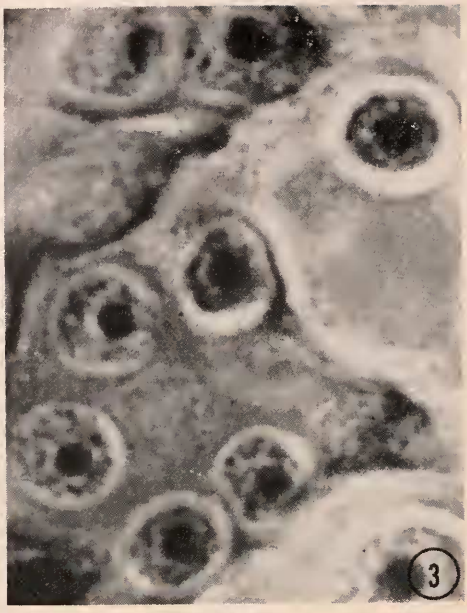


FIGURE 1. Sagittal view of *Rhodnius* ovariole.

PLATE II





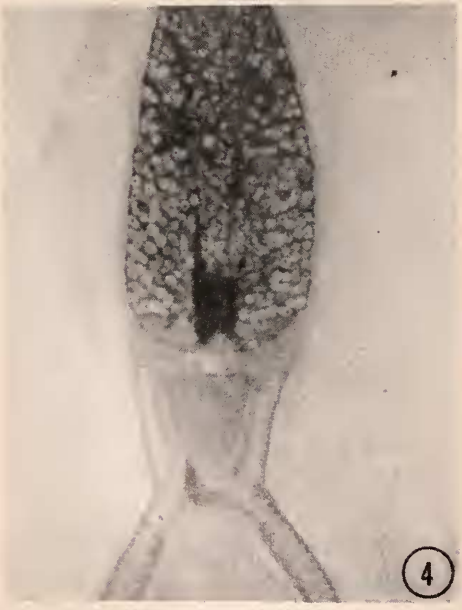


FIGURE 2. Zone I cells of apical trophic tissue. The large nuclei contain peripheral chromatin granules. Heidenhain's hematoxylin. Approximately 1250 ×.

FIGURE 3. Zone II of apical trophic tissue. The enlarged nuclei now have centrally located nucleoli, and are located in clumps of cytoplasm. Heidenhain's hematoxylin. Approximately 1325 ×.

wax double embedding technique was utilized to prepare serial sections of 5- to 7-micron thickness. These sections were stained with Heidenhain's iron hematoxylin. They were differentiated in a saturated solution of picric acid in 70% alcohol, and no counterstain was employed.

B. Histochemical methods

The localization of DNA was accomplished by the Feulgen method used as follows. Carnoy-fixed sections were hydrolyzed in 1.0 N HCl at 60° C. for 8 minutes, while adjacent sections were placed in distilled water as controls. Sections were then rinsed in distilled water and transferred to the Schiff reagent at room temperature for one hour. They were then rinsed in three changes (10 minutes each) of freshly prepared bleaching solution consisting of 5 ml. of 10% aqueous $K_2S_2O_5$, 5 ml. of 1.0 N HCl, and 90 ml. of distilled water. This was followed by washing in tap water for 5 minutes, dehydration, clearing, and mounting in balsam.

RNA was detected by staining Carnoy-fixed sections with a potassium acid phthalate-buffered solution of Azure B bromide following exactly the procedure developed by Flax and Himes (1952). Adjacent control sections were pretreated with ribonuclease[3] (0.5 mg./ml. glass-distilled water) at 37° C. for two to four hours, while the test sections were incubated in distilled water at the same temperature.

[3] Obtained from Worthington Biochemical Laboratories, Freehold, N. J.

C. Autoradiographic methods

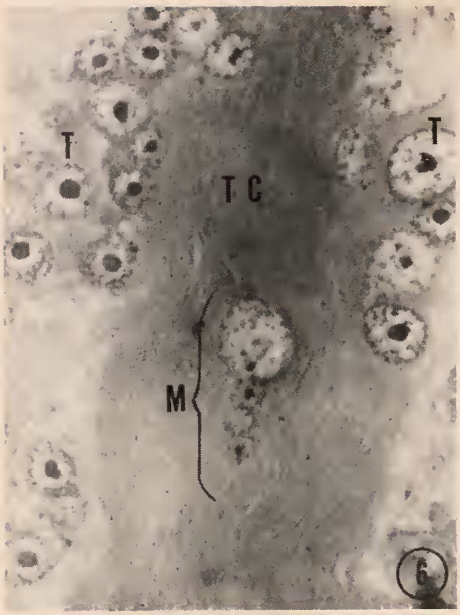
The autoradiographic localization of sites of incorporation of tritium-labeled precursors into DNA, RNA, and protein was determined as follows. The bugs being studied were each injected with 5 microcuries of thymidine-H^3 (1900 mc./m*M*) for determination of DNA synthesis, 5 microcuries of uridine-H^3 (3280 mc./m*M*) for determination of RNA synthesis, or 10 microcuries of DL-leucine-4,5-H^3 (3570 mc/m*M*) for determination of protein synthesis. The radioactive solutions were so diluted that each bug received 0.01 ml. per injection.

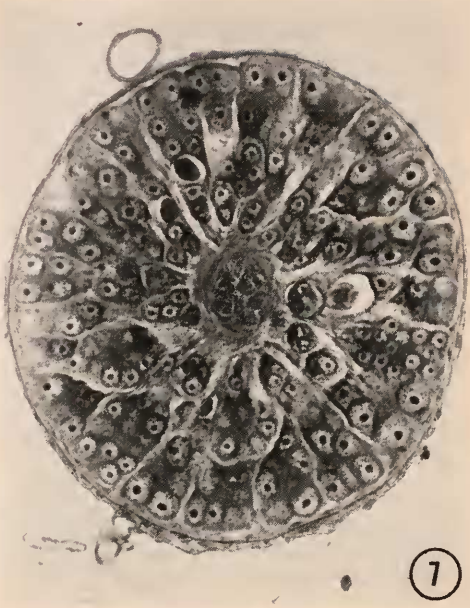
An uptake time of 15 minutes to two hours was allowed, after which the bug was dissected. The tissues were fixed for 45 minutes in Carnoy's solution, hydrated and then thoroughly washed in running water to remove the unincorporated labeled precursors. Using the histological procedures previously described, sections were cut at 7 microns and affixed to glass slides. The slides with hydrated tissues were then washed in water to ensure removal of any remaining labeled precursor. Kodak Nuclear Track Bulk Emulsion (Type NTB-3) was liquefied in a 43° C. water bath, and each slide was dipped in the emulsion and allowed to gel in a vertical postion in a darkroom maintained at 15° C. After

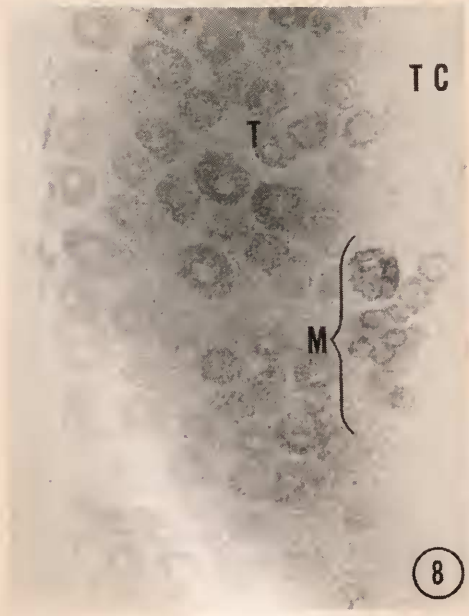
FIGURE 4. Germarium and upper portion of vitellarium showing cytoplasmic basophilia. Azure B bromide. Approximately 100 ×.

FIGURE 5. Section of different ovariole from the same ovary used for Figure 4. Note the loss of cytoplasmic basophilia after two hours of ribonuclease incubation. Nucleolar basophilia still persists in most of the trophocytes. Longer treatment with ribonuclease yields slides that are completely blank and non-photogenic. Azure B bromide. Approximately 100 ×.

PLATE III







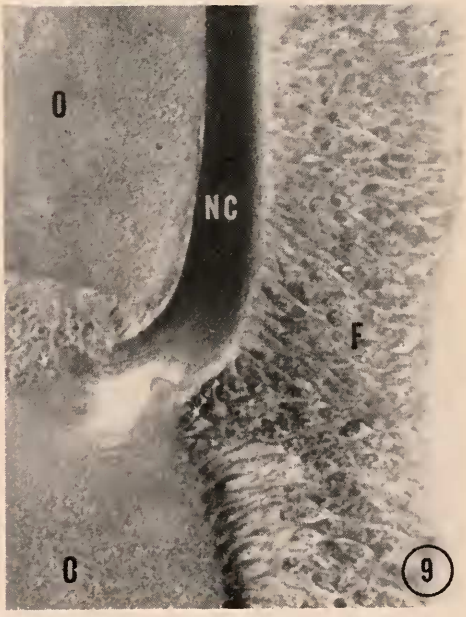


FIGURE 6. Migratory nuclei (M) in trophic core (TC) of Zone III of apical trophic tissue. (T) trophocyte nuclei of Zone III. Heidenhain's hematoxylin. Approximately 800 ×.

FIGURE 7. Zone III of germarium. Cross-section showing masses of cytoplasm projecting radially into trophic core. Heidenhain's hematoxylin. Approximately 250 ×.

drying, the slides were placed in light-tight Bakelite boxes containing Drierite, and allowed to expose for periods of two to four weeks at 3° C. The slides were then developed in Kodak Dektol for 1½ minutes, fixed for 10 minutes in Kodak acid fixer, and washed in several changes of water for 15 minutes. The sections were stained with Azure B bromide as previously described, dehydrated, and mounted in balsam under a cover glass. When viewed under a microscope, those areas of the emulsion with granulation denser than the background presumably lie above areas of the section which were sites of incorporation of the labeled precursor into the macromolecule being studied.

In subsequent experiments, after the initial ½-hour uptake of the labeled precursor, the bug was injected with 100 times the concentration of the same precursor in its unlabeled form. This had the effect of diluting the labeled material to an extent that one could assume only negligible radioactive uptake after this second injection. Bugs so treated were killed and fixed at various time intervals, thus allowing the tracing of the ½-hour "pulse" of radioactivity through the cells.

Results

A. Histology and histochemistry of the ovary

Each ovary of *Rhodnius* consists of seven telotrophic ovarioles. Apically, each ovariole tapers to form a long terminal filament. Immediately posterior to the terminal filament is a lanceolate structure, the germarium, which contains apical trophic tissue, young oocytes, and prefollicular tissue. Posterior to the germarium is the vitellarium, consisting of a string of growing oocytes, each one surrounded by follicular cells. The entire ovariole is enclosed in a membrane, the tunica propria. After the chorion is deposited, the mature oocyte is released from its follicle into the pedicel of the ovariole, from which it passes into the lateral oviduct. Figure 1 is a diagram of a sagittal section of the germarium and vitellarium.

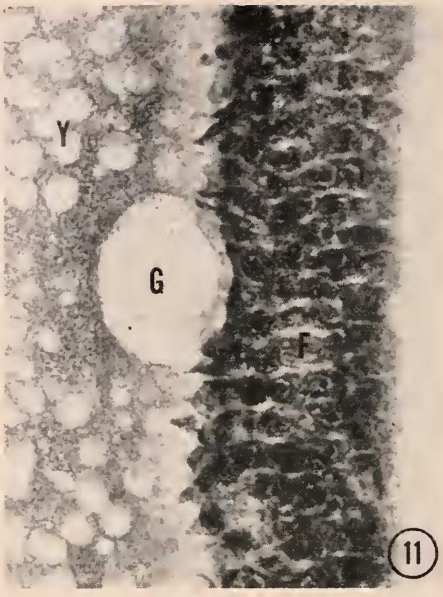
The apical trophic tissue of the germarium may be divided into three zones similar to the zones described by Bonhag (1958) for *Oncopeltus*. In Zone I (Fig. 2), the trophocytes (nurse cells) have distinct boundaries. Each cell contains a single large nucleus with peripheral Feulgen-positive chromatin granules. Occasional mitoses are observed in the region.

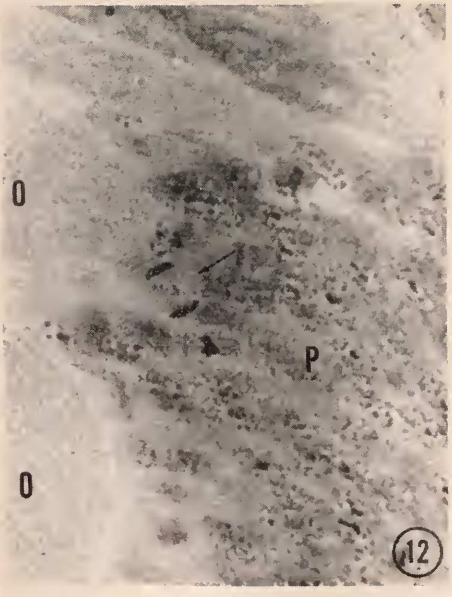
Zone II is the transitional zone of trophocyte differentiation. It is marked by the disappearance of individual cell boundaries, the enlarged nuclei coming to lie in clumps of cytoplasm (Fig. 3). Each nucleus possesses a large, centrally located basophilic nucleolus. The Feulgen granules arranged around the periphery of each nucleus are much more intense than those of Zone I, suggesting that these cells are polyploid. The cytoplasm of this region is strongly basophilic. The complete loss of this basophilia after two hours of ribonuclease treatment indicates the presence of RNA (Figs. 4 and 5). The nucleolar basophilia is

Figure 8. Feulgen-stained portion of Zone III of germarium showing (T) trophocytes with Feulgen-positive granules in nuclei, but with Feulgen-negative nucleoli; (M) migratory nuclei in various stages of fusion and breakdown; (TC) trophic core. Approximately 800 ×.

Figure 9. Nutritive cord (NC) leading to oocyte (O). (F) follicle cells. Heidenhain's hematoxylin. Approximately 600 ×.

PLATE IV





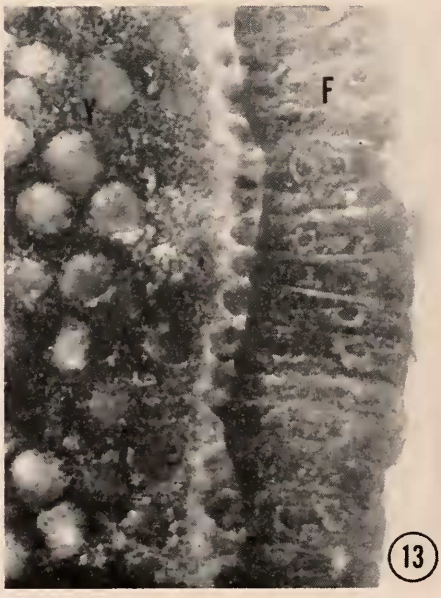


FIGURE 10. Stages in growth of oocytes. Heidenhain's hematoxylin. Approximately 375 ×.

FIGURE 11. Germinal vesicle (G) in close proximity to cells of follicular epithelium (F). (Y) yolk. Heidenhain's hematoxylin. Approximately 675 ×.

more persistent, requiring at least four hours of ribonuclease incubation before it is completely lost.

The processes of nuclear aggregation within common cytoplasmic masses continue into Zone III. The nuclei in this region of the germarium are arranged around a central cylinder of cytoplasm, the trophic core. This core appears fibrous in structure, and stains strongly with Azure B bromide (Figs. 4, 5 and 6).

The trophocyte nuclei are imbedded in fingerlike masses of cytoplasm which project radially into the trophic core (Fig. 7). The nuclei appear to be proliferated from these masses, eventually passing into the core. The appearance of migratory nuclei of different sizes within the core (Fig. 6) suggests that some of these nuclei have become fused. The migratory nuclei can be seen in what appear to be stages of degeneration and breakdown, leading to the release of Feulgen-positive material into the core (Fig. 8).

The posterior end of the trophic core connects with the young oocytes by means of cytoplasmic strands, the nutritive cords (Fig. 9). These cords have the same histological and histochemical characteristics as the trophic core. Although present in the posterior portion of the trophic core, neither broken down portions of the migratory nuclei, nor Feulgen-positive material were ever observed in the cords leading to the oocytes.

The oocytes get progressively larger as they pass down from the base of the germarium through the vitellarium (Fig. 10). The germinal vesicle, which also enlarges during oocyte growth, contains Feulgen-positive granules, in addition to basophilic nucleoli-like structures that disappear after ribbonuclease treatment. Vacuoles are generally present. The germinal vesicle is often found in close proximity to the follicular epithelium (Fig. 11). The oocyte yolk, which stains strongly with Azure B bromide during the early stages of development, diminishes in the intensity of its basophilia as the oocyte grows.

Immediately posterior to the apical trophic tissue, and surrounding the young oocytes, is a region of prefollicular tissue (Fig. 12). This is a zone of active mitosis. Cells proliferated from this mass of tissue become oriented around the enlarging oocytes as they pass down the vitellarium. These follicle cells surrounding the young oocytes continue mitotic division during the early stages of oocyte growth.

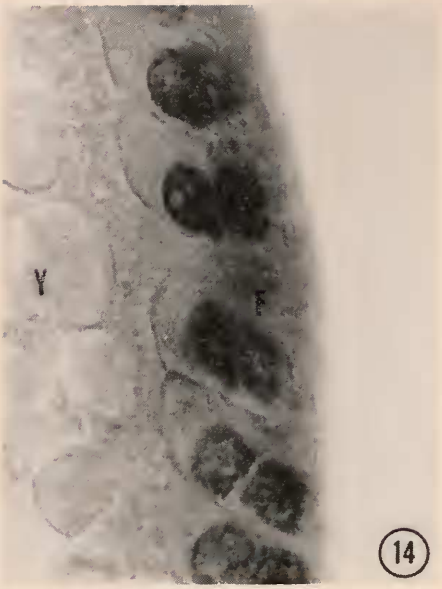
When the oocyte reaches a size of about 0.4 mm. in diameter, the follicle cells surrounding it undergo amitosis and become binucleate. The follicular epithelium at this stage is made up of tightly packed columnar cells (Fig. 13). As the process of oocyte growth continues, the follicle cells become cuboidal. Each nucleus gives an intense Feulgen reaction, suggesting that it is polyploid (Fig. 14). The strongly basophilic cytoplasm, with no staining in the ribonuclease controls, indicates the presence of RNA (Figs. 15 and 16).

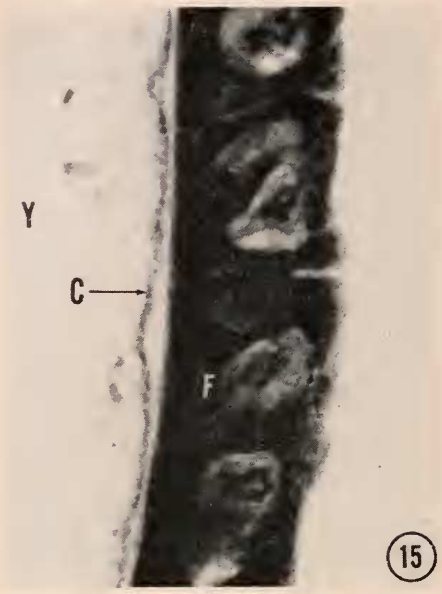
After the completion of yolk deposition, the follicle cells lay down the various layers of the chorion and then by means of prominent villi imprint upon it the characteristic surface sculpturing (Fig. 17). The cytology of the follicular

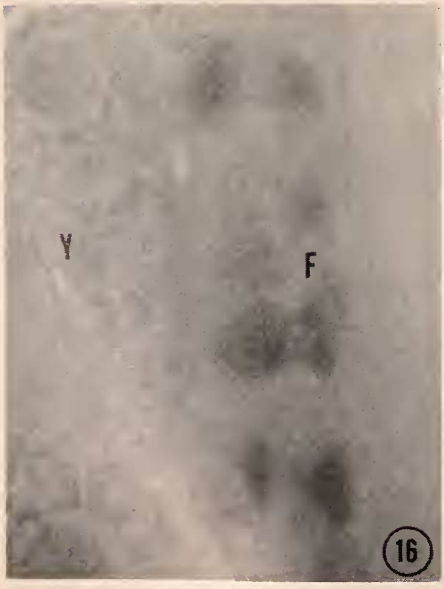
FIGURE 12. Region of prefollicular tissue (P) surrounding young oocytes (O). Note the anaphase (arrow) in the prefollicular tissue. Heidenhain's hematoxylin. Approximately 800 ×.

FIGURE 13. Columnar stage of follicular epithelium (F) surrounding yolk (Y) of oocyte. Each follicle cell is binucleate. Heidenhain's hematoxylin. Approximately 675 ×.

PLATE V







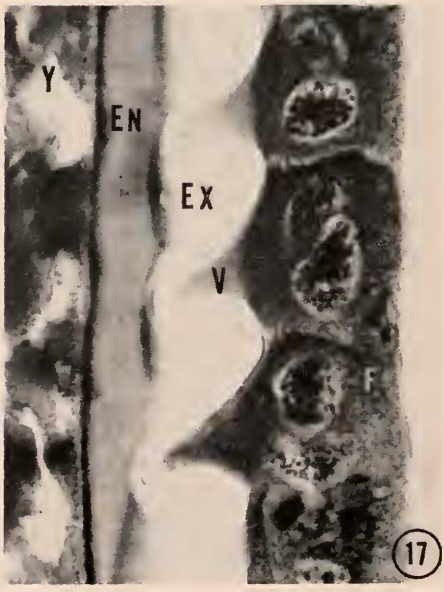


FIGURE 14. Cuboidal stage of follicular epithelium (F) with Feulgen staining. All of the nuclei of the follicle cells are Feulgen-positive. (Y) yolk. Approximately 850 ×.

FIGURE 15. Follicle cells (F) and yolk (Y) of oocyte, showing basophilia in cytoplasm, in nuclear granules of the follicle cells, and in the cortical cytoplasm (C) of the yolk. Azure B bromide. Approximately 850 ×.

epithelium, and the process of chorion deposition in *Rhodnius* have been described in detail by Beament (1946).

B. Autoradiographic results

1. DNA. The incorporation of tritiated thymidine was restricted primarily to nuclei. After 15 minutes of thymidine uptake, it was possible to detect granules in the emulsion above the nuclei of all types of ovarian tissue. Trophocyte nuclei (Fig. 18) in all portions of the germarium, as well as migratory nuclei, were observed to be engaged in thymidine incorporation. The follicular tissue in its various stages, from the prefollicular region to the cuboidal follicular region in the process of secreting the exochorion, showed a higher rate of thymidine incorporation than any other tissue. The tight packing of the young follicle cells resulted in a high density of nuclei per tissue section, thus making it difficult to localize individual clumps of granules above specific nuclei (Fig. 19). However, general labeling was found above the nuclear region of the cells, and there was little if any cytoplasmic labeling in this region. In addition, there was no indication of thymidine incorporation within the nutritive cords, or of the early passage of labeled material down the cords (Fig. 19). Some granules were apparent above the cytoplasmic region of the older follicle cells. The great preponderance of incorporation, however, was nuclear (Fig. 20). No granules were observed above the germinal vesicle of any stage. In addition to ovarian labeling, incorporation was observed in the nuclei of the fat body cells (Fig. 21) and the midgut cells.

This process of predominant nuclear incorporation of thymidine into DNA and retention of the DNA by the nuclei continued for up to 4 to 6 hours. At this time it became possible to detect the appearance of granules above the cytoplasm of the ovarian cells. The spread of radioactivity continued gradually, after 6 to 8 hours extending over the yolk. Unfortunately, under the conditions of the experiment, the time sequence of the label migration did not show a clear progression from the nuclei to the cytoplasm and then to the yolk *via* the nutritive cords. There was instead a gradual leakage which occurred in all regions of the ovary and was rather difficult to trace.

2. RNA. The pattern of incorporation of labeled uridine into RNA showed strong nuclear labeling in the trophocytes (Fig. 22) and in the follicle cells (Fig. 23). The three zones of the apical trophic tissue contained labeled cells, but apparently incorporation occurred in an asynchronous manner, for some cells gave no evidence of uridine uptake. This was in contrast to the situation in the follicle cells, where all nuclei were found to be heavily labeled in all regions from the prefollicular zone down to the follicular epithelial cells just before the process of exochorion deposition.

Ovaries fixed 30 minutes after administration of the uridine showed no label in the mature follicle cells which had completed exochorion formation. When

FIGURE 16. Section adjacent to the one shown in Figure 15, demonstrating the loss of basophilia after two hours of ribonuclease incubation. (F) follicular epithelium; (Y) yolk. Azure B bromide. Approximately 850 ×.

FIGURE 17. Follicle cells (F) in process of imprinting the surface sculpturing on the chorion of the mature oocyte. (V) villus of follicle cell; (EX) exochorion; (EN) endochorion; (Y) yolk. Heidenhain's hematoxylin. Approximately 850 ×.

PLATE VI

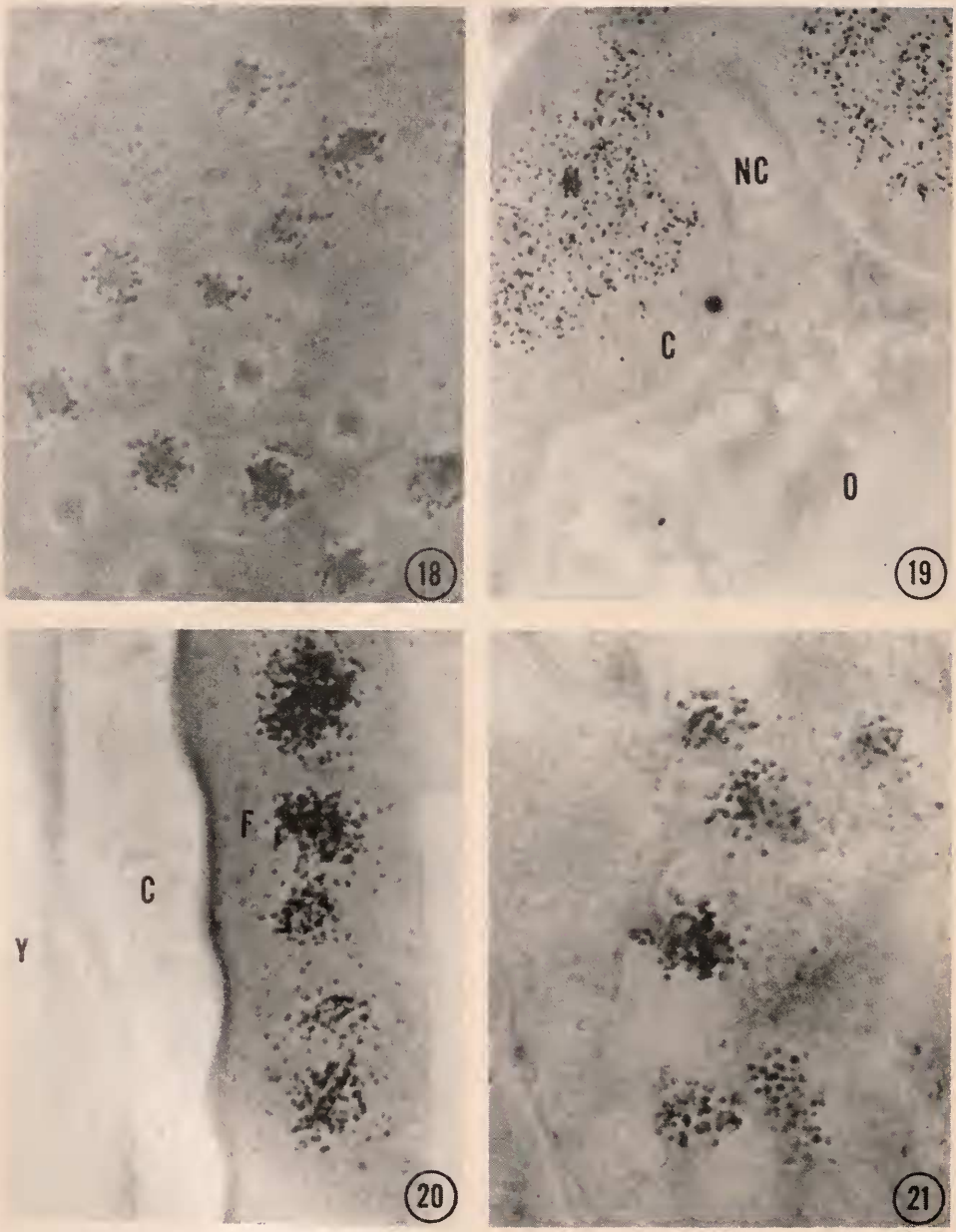


FIGURE 18. Granules above nuclei of trophocytes. Tissue fixed one hour after injection of H^3-thymidine. Approximately 1050 ×.

FIGURE 19. Granules above tightly packed nuclei (N) of young follicular epithelium. No granules are present, at this time, above the cytoplasm (C) of the follicle cells, above the

the 30-minute "pulse" of labeled uridine was followed, the anteriormost of these exochorion-follicle cells gave evidence of labeling in 4 to 6 hours, indicating the rate of movement of the cells and of the oocyte with which they were associated. At some time from 16 to 48 hours, the movement of the follicle cells had progressed to the point where all of the mature follicle cells were labeled.

In following the intracellular movement of label, there was indicated after one to two hours a gradual drift-out from all nuclei (Figs. 24 and 25) into the cytoplasm. This movement continued over a period of time until after 12 hours the cytoplasm was uniformly labeled. There was also a transfer of some of the label to the yolk during this period. The same sequence of uridine incorporation and RNA movement occurred in the fat body and in the midgut, though the midgut rate of incorporation was considerably lower. No incorporation was noted in the germinal vesicle.

3. Protein. The study of protein synthesis, as indicated by the early incorporation of labeled leucine, showed an even distribution of granules above the nuclei and cytoplasm of the germarium (Fig. 26). A much heavier granulation was found above all stages of the follicular tissue (Fig. 27). In addition, the emulsion above the nuclei and cytoplasm of the fat body and midgut contained evenly distributed grains of reduced silver. No granules were noted above the germinal vesicle at any stage.

After two hours it was possible to detect a transfer of some of the label in the follicle cells to the oocyte yolk (Fig. 28). This process continued over a period of several hours. The proteinaceous components of the chorion were found to be deposited in discrete layers, as indicated by Beament (1946), though the autoradiographic timing methods used were not precise enough to distinguish all of the seven chorion layers that Beament described. Figure 29 shows heavy granulation above the layer which is here interpreted as the soft exochorion layer of Beament.

Discussion

A. DNA

The evidence indicates that the trophic tissues of the *Rhodnius* ovary are active in transferring DNA in a partially depolymerized form to the growing oocyte. The breakdown of migratory nuclei in the germarium releases Feulgen-positive material into the trophic core, yet no droplets can be found in the strands leading to the oocytes. Processes similar to this have been reported by Schrader and Leuchtenberger (1952) for the coreid bug, *Acanthocephala*, and by Bonhag (1955) for the milkweed bug, *Oncopeltus*. It is conceivable that the failure to detect DNA in the nutritive cords and in the oocyte cytoplasm by use of the Feulgen technique is a result of the great dilution that the DNA would undergo when released. It would seem more reasonable, however, that the DNA is depolymerized to some Feulgen-negative form before being transferred to the oocyte.

nutritive cord (NC), or above the oocyte (O). Tissue fixed one hour after injection of H^3-thymidine. Approximately 650 ×.

Figure 20. Granules predominantly above nuclei of follicular epithelium (F). No granules are present above the chorium (C), or above the yolk (Y). Tissue fixed one hour after injection of H^3-thymidine. Approximately 1250 ×.

Figure 21. Granules above nuclei of fat body cells. Tissue fixed one hour after injection of H^3-thymidine. Approximately 1500 ×.

PLATE VII

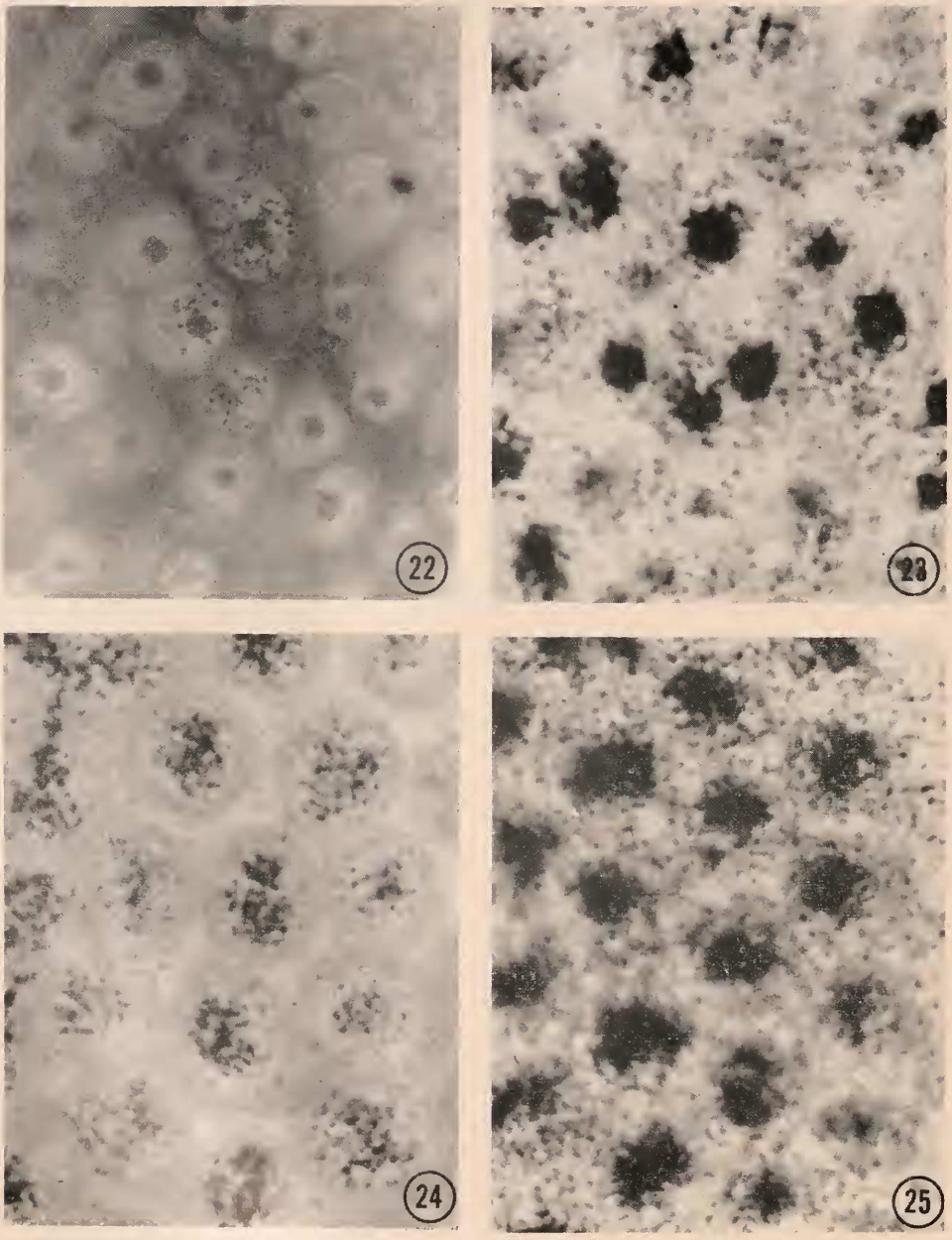


FIGURE 22. Granules above nuclei of trophocytes. Thirty-minute uptake of H^3-uridine allowed before radioactive precursor was diluted out. Tissue fixed one hour after H^3-uridine injection. Approximately 1050 ×.

FIGURE 23. Granules predominantly above nuclei of trophocytes, with some granulation above cytoplasm. Thirty-minute uptake of H^3-uridine allowed before radioactive precursor was diluted out. Tissue fixed two hours after H^3-uridine injection. Approximately 1050 ×.

The autoradiographic results tend to support this conclusion. The nonspecific spread of granules through the cytoplasm of the germarium, when the time course of the tritiated thymidine pulse is studied, suggests the passage of a diffusible form of DNA from nuclei to cytoplasm and eventually into the cytoplasm of the growing oocyte. The autoradiographs indicate that this process also takes place in the follicle cells. At the same time that the label is observed moving from the nuclei of the follicular epithelium to the cytoplasm, the Feulgen test shows that the intact DNA of the follicle cell is retained exclusively in the nuclei. Here again, a depolymerization of DNA into a diffusible Feulgen-negative form is suggested.

Nigon and Nonnenmacher (1961) have reported the incorporation of tritiated thymidine into the cytoplasm as well as the nuclei of nurse cells in the *Drosophila* ovary. However, their flies were studied an hour after administration of the precursor, and it is conceivable that their cytoplasmic label represents migration from the nucleus to the cytoplasm during the one-hour uptake period, rather than the cytoplasmic incorporation that they postulated. Similarly, Durand (1958) has shown that in the ovary of *Gryllus,* the cytoplasm of the mature follicle cells, as well as the basophilic network of the oocyte, show evidence of the presence of a label after the administration of tritiated thymidine. Granules were found above the cytoplasmic villi of the follicle cells, strongly suggesting the migration of the label from the follicle cells to the oocyte. However, no pulse type experiments were performed in either of these studies in an attempt to determine the time sequence of the label incorporation and migration. Consequently, there was no way of distinguishing sites of incorporation from sites of storage after migration. Jacob and Sirlin (1959) have presented autoradiographic evidence for the loss of DNA from the trophic tissue of the *Drosophila* ovary, and the transfer of either DNA or acid-soluble nucleosides to the ooplasm.

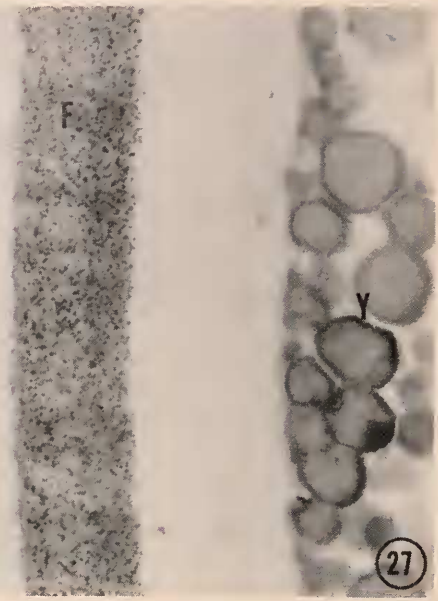
Zalokar (1960b) has discussed the theoretical considerations involved in the autoradiographic determination of the site of formation of a substance from its precursor, as opposed to the determination of the intermediary storage site of the synthesized substance. He concluded that if one is interested in localizing the site of formation, that in addition to utilizing a precursor of high specific activity in a high concentration, the time between the administration of the precursor and the immobilization of the product should be as short as possible, never substantially exceeding the time required to synthesize a complete molecule of the product.

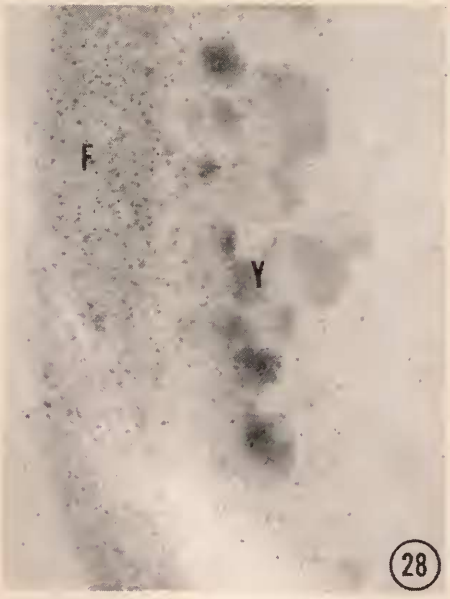
In the present experiment, granules indicating the incorporation of tritiated thymidine were detected exclusively above the nuclei of nurse and follicle cells 15 minutes after injection of the thymidine. This was a substantially longer time than that recommended by Zalokar. Nevertheless, on the basis of the timed pulse experiments, it seems reasonable to assume that the thymidine was incorporated in the nuclei, and migrated to the cytoplasm in a form which was depolymerized sufficiently that it gave a Feulgen-negative test. Furthermore, the migrating labeled material was insoluble enough to be retained in the tissues after washing

FIGURE 24. Granules above nuclei of follicular epithelium (tangential section). Thirty-minute uptake of H^3-uridine allowed before radioactive precursor was diluted out. Tissue fixed one hour after H^3-uridine injection. Approximately 1100 ×.

FIGURE 25. Granules predominantly above nuclei of follicular epithelium, with some granulation above cytoplasm (tangential section). Thirty-minute uptake of H^3-uridine allowed before radioactive precursor was diluted out. Tissue fixed two hours after H^3-uridine injection. Approximately 1100 ×.

PLATE VIII





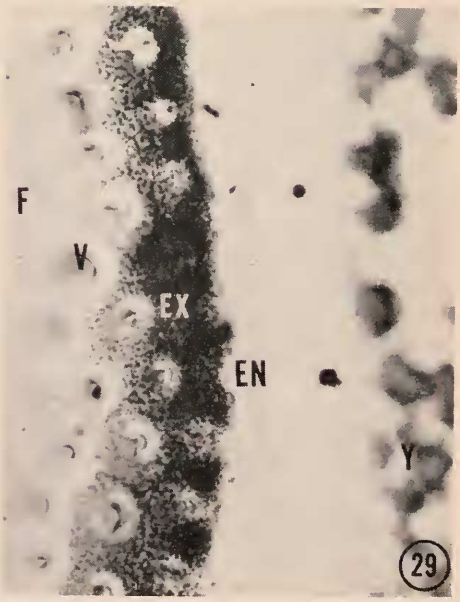


FIGURE 26. Granulation above nuclei and cytoplasm of germarium. Tissue fixed thirty minutes after injection of H^3-leucine. Approximately 300 ×.

FIGURE 27. Granulation above nuclei and cytoplasm of follicular epithelium (F), with no granules above yolk (Y) of oocyte. Tissue fixed thirty minutes after injection of H^3-leucine. Approximately 550 ×.

with reagents as previously described, or was protected from the action of the histological reagents by an association with proteins denatured during fixation.

One is struck by the increase in DNA content in the nuclei of both the apical trophic tissue and the follicular epithelium. In the nurse cells there is a distinct increase in the intensity of the Feulgen staining from the nuclei of the first through the third zones. On the basis of microspectrophotometric measurements of Feulgen staining in the ovary of the bug, *Acanthocephala*, Schrader and Leuchtenberger (1952) concluded that the irregular increase in DNA content of the more distal nuclei could best be explained by a process of fusion. In *Rhodnius*, the incorporation of thymidine in all zones of the germarium makes it apparent that endopolyploidy is an important factor in the increase, though cytological study suggests that fusion of nuclei also occurs.

The uptake of thymidine by the prefollicular tissue and by the young follicular epithelium is probably associated with the active mitosis that occurs in these tissues. However, after the follicle cells become binucleate, no further mitoses are observed. Inasmuch as the nuclei continue to incorporate thymidine and increase in Feulgen staining intensity during this period, it is apparent that endopolyploidy is occurring.

One can only speculate on the significance of the considerable rate of synthesis of DNA by the trophic tissues of the ovary, and the apparent transfer of some of this DNA to the growing oocytes. The phenomenon appears to be widespread in insects. Kaufmann *et al.* (1953) reported that in the ovary of the wasp, *Habrobracon*, polyploid nurse-cell nuclei disintegrate and are engulfed by the egg cytoplasm, with the DNA from the nurse-cells being quickly broken down and dispersed throughout the egg. The authors concluded that DNA building blocks are probably stored in the egg cytoplasm. Durand (1955) has shown that the unfertilized egg of *Gryllus* contains 1600 times more DNA than the spermatozoan. A reserve of DNA or of DNA precursors has been found in the egg of *Drosophila* by Levenbook *et al.* (1955), and by Nigon and Daillie (1958).

It seems unlikely that any genetic information is carried over in the transfer of DNA from the maternal ovary to the developing egg. It would appear more reasonable that the reserve of DNA or of its precursors serves as a readily available source of building blocks to supply the needs of the cleavage nuclei.

All of the excess DNA synthesized in the ovary, however, is not utilized for this egg reserve. The follicle epithelial nuclei of *Rhodnius* continue synthesizing DNA even after the process of chorion deposition over the oocyte has been completed, and any further transfer of material between the follicle cell and the oocyte would be impossible. The apparent overproduction of DNA is a phenomenon which is not unknown in insects. Wigglesworth (1942, 1948) has shown that during the height of cell division in the epidermis of *Rhodnius* and other insects, mitosis is so exuberant that many more cells are produced than will be needed

FIGURE 28. Granulation above follicular epithelium (F), and above yolk (Y). Thirty-minute uptake of H^3-leucine allowed before radioactive precursor was diluted out. Tissue fixed six hours after injection of H^3-leucine. Approximately 550 ×.

FIGURE 29. Section of mature oocyte cut almost on a tangent. This has the effect of stretching out the horizontal dimension of the section. (F) follicle cells; (V) villus of follicle cell; (EX) exochorion; (EN) endochorion; (Y) yolk. Granulation is heavy above the exochorion. Tissue fixed four hours after injection of H^3-leucine. Approximately 500 ×.

to form the new integument. The unwanted cells die, releasing Feulgen-positive droplets which are apparently assimilated by the neighboring cells.

B. RNA

Clearly, both the trophocytes and the follicular epithelial cells function in the transfer of RNA to the oocyte of *Rhodnius*. The histochemical evidence for the passage of RNA from ovarian trophic tissues of insects to the growing oocytes has been reviewed by Bonhag (1958). The histochemical observations on *Rhodnius* are in accord with the previous findings on the apparent passage of RNA from the trophocytes through the RNA-rich nutritive cords in telotrophic ovaries. The *Rhodnius* ovariole exhibits a reduction in the intensity of oocyte basophilia due to RNA as the oocyte enlarges. Because of the enormous amount of yolk added to the oocyte during growth, it is likely that this reduction in basophilia is caused by a dilution of the RNA rather than by a reduction in it.

The present autoradiographic studies have confirmed the observations on the transfer of RNA from the trophocytes, and extended these findings to the follicle cells. The autoradiographic observations on the intracellular movement of RNA are consistent with the results of King and Burnett (1959), Zalokar (1960a), and Sirlin and Jacob (1960), demonstrating the incorporation of labeled precursors into the RNA of the nurse cell and follicle cell nuclei of *Drosophila,* with the subsequent movement of the label to the cytoplasm.

Inability to demonstrate uridine incorporation in the mature follicle cells which have completed the process of exochorion deposition is of particular interest. On the basis of the hypothetical function of the RNA, this is what might have been expected. One might conceive of the nuclei of the trophic tissue being involved in the synthesis of at least two functionally different classes of RNA. First of all, we might expect the synthesis and transfer of RNA, capable of transmitting genetic information which regulates the formation of oocyte protein by the cytoplasm of the trophic tissue. Presumably, the protein components of the exochorion are the last proteins transferred to the oocyte by the follicle cells before the latter are autolyzed. There would thus be no requirement for further synthesis of this type of informational RNA after the formation of the exochorion.

Second, some of the RNA produced by the trophic tissues is transferred, as has been shown, to the oocyte, where it may serve as a reserve for the developing embryo. The further transfer of this RNA to the oocyte would be impossible after the formation of the chorion.

C. Protein

The study of the 30-minute pulse of incorporation of tritiated leucine clearly indicates the passage of protein from the trophic tissues to the oocyte. Synthesis appeared to take place throughout the cytoplasm and nuclei of all the trophic cells. King and Burnett (1959), studying the uptake of tritiated glycine by *Drosophila* ovaries, also reported amino acid incorporation by both cytoplasm and nuclei. Exclusive incorporation in the cytoplasm of the *Drosophila* ovary during the first minute after injection of tritiated leucine, however, was noted by Zalokar (1960a). After 16 minutes, the nuclei became as heavily labeled as the cytoplasm.

Incorporation of tritiated histidine into the nurse and follicle cells of *Calliphora* was shown to be predominantly cytoplasmic during the first 15 minutes after injection, by Bier (1962). It seems possible that the nuclear label in the present experiment represented migration from the cytoplasm rather than the initial site of protein synthesis. Results similar to those of Zalokar and Bier might have been obtained if the *Rhodnius* ovaries had been fixed a short time after injection, instead of after 30 minutes.

In *Rhodnius,* the greatest increase in oocyte size is observed after the oocyte has been separated from the nutritive cords, and the responsibility for nutrition has been taken over by the follicular epithelium. Because of this, and because of the fact that the number of reduced silver grains is considerably greater in the emulsion above the follicle cells than above the trophocytes, it is obvious that the follicular epithelium plays a more important role in the transfer of proteins to the oocyte.

The large number of reduced silver grains above the fat body indicates that it was almost as active in protein synthesis as the follicular epithelium, the two tissues showing considerably more amino acid incorporation than any other tissues studied. It is probable that much of the protein synthesized by the fat body is transferred to the blood and plays a role in yolk formation. Shigematsu (1958) has reported the *in vitro* incorporation of labeled amino acids into the larval fat body of the silkworm, *Bombyx,* with the subsequent release of synthesized protein into the incubating medium. Wigglesworth (1943) has suggested that much of the normal protein in the yolk may be synthesized elsewhere and merely transferred to the egg by the follicle cells. Evidence for this process taking place in the silkworm, *Samia,* has been obtained by Laufer (1960a, and 1960b) by means of zone electrophoresis of tissue proteins in starch. A female-specific protein is present in high concentration in the fat body, which seems to be the source of this protein. Laufer has been able to demonstrate the appearance of the protein in the blood, and later in the eggs. This is consistent with the immunochemical observations of Telfer (1954, 1960), who demonstrated that a number of antigens present in the blood of the Cecropia moth were accumulated by the oocytes. He also found that several oocyte antigens could not be detected in the blood. These latter antigens may have represented proteins that were synthesized in the ovary.

There is thus evidence from various sources that the synthesis of oocyte protein may be both ovarian and extra-ovarian. That both processes may take place in *Calliphora* has been shown autoradiographically by Bier (1962). Ovarian synthesis of protein is detectable one to 15 minutes after injection of tritiated histidine. Forty minutes after injection of the precursor, a protein, apparently synthesized outside the ovary, accumulates at the periphery of the ooplasm and migrates into the oocyte in the form of rounded platelets. No clear distinction between these two processes was observed in the present study. The transfer of label from the follicular epithelium to the interior of the oocyte was instead a slow and uniform process, with no indication of either the accumulation of protein at the periphery of the oocyte, or of the inward migration in the form of platelets. There is some circumstantial evidence, however, that suggests that much of the protein synthesized by the *Rhodnius* fat body may be utilized by the ovaries.

Allatectomized bugs, having little oocyte development, show only negligible amino acid uptake by the fat body. On the other hand, control bugs, with full ovarian development, reveal that the fat body is active in amino acid incorporation. It is probable that the transfer of label from the follicle cells to the oocytes in the present study represents a summation of both processes: follicular epithelial synthesis, and extra-ovarian synthesis.

Bonhag (1958) has reviewed the literature on the formation of yolk protein in insect ovaries. At one time or another, practically every cellular structure known to the light microscopist has been reported to produce or to transform into yolk protein. These conclusions have generally been based on cytological evidence so tenuous as to be of limited value. It is quite evident from the present studies that in *Rhodnius,* at least, most of the yolk protein is transferred to the oocyte from the follicle cells. How much of the protein is actually synthesized by the follicular epithelium, and how much of it is merely transferred to the epithelium from the blood is difficult to determine. Contrary to many of the previous reports on vitellogenesis, as cited by Bonhag, the germinal vesicle appears to play only a minor role in the synthesis of protein.

Summary

1. Histochemical and autoradiographic techniques were applied to *Rhodinus prolixus* during vitellogenesis.

2. Examination of the ovary, fat body, and midgut demonstrated that DNA was synthesized exclusively in the nuclei of these tissues. There appeared to be a transfer of some of the DNA in a partially depolymerized form from the trophic tissues of the ovary to the growing oocyte.

3. RNA was synthesized in the nuclei of the ovary, fat body, and midgut, and subsequently was transferred to the cytoplasm of these tissues. Some of the newly synthesized RNA passed from the trophic tissues of the ovary to the enlarging oocyte.

4. Protein was found to be synthesized most actively in the follicular epithelial tissues of the ovary, and in the fat body. Evidence of protein synthesis was also found in the other regions of the ovary, and in the midgut. The passage of newly synthesized protein from the follicular epithelium into the oocyte was noted. Synthesis of yolk protein by the oocyte itself appeared to be negligible.

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